

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Mueller, Rainer, *et al.*

Application No.: To Be Assigned

Group No.: To Be Assigned

Filed: July 23, 2001

Examiner: To Be Assigned

For: EXPRESSION OF ALKALINE PHOSPHATASE IN YEAST

Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

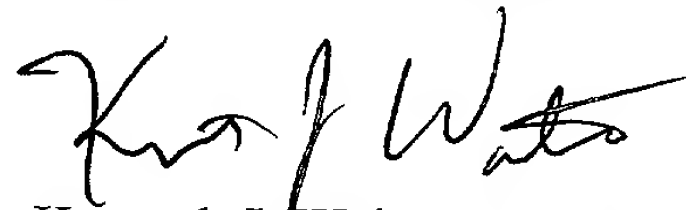
Sir:

Please enter the following amendments prior to examination of the above-referenced application:

IN THE CLAIMS:

Please enter the following amendments to claims 1 through 15 as filed in the originally filed application. Both a clean and a marked-up copy of the claims as amended are attached.

Respectfully submitted,



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Claims

1. A process for the production of a eukaryotic alkaline phosphatase in yeast cells comprising the steps: a) cloning [a] an alkaline phosphatase gene sequence into different vectors b) transformation of the yeast, c) expression of the alkaline phosphatase and d) purification of the alkaline phosphatase, wherein
 - a first vector is utilized that has a resistance gene for a first selection marker,
 - transformants are used which have integrated the resistance gene and the gene sequence into the genome, said transformants being [are] selected by growth on nutrient medium containing a low concentration of a first selection marker,
 - the gene copy number is increased by multiple transformation in which multiple transformants are selected by growth on nutrient medium at an increased selection pressure,
 - a second vector is [added] used which has a resistance gene for a second selection marker,
 - the gene copy number is increased by multiple transformation with the second vector in which multiple transformants are selected by growth on nutrient medium at an increased selection pressure and
 - those [clones] transformants [are selected] which have integrated several copies of the gene sequence and the selection marker resistance genes in a stable manner are selected for producing the eukaryotic alkaline phosphatase.
2. The process according to claim 1 [the invention], wherein the gene sequence for the alkaline phosphatase corresponds to SEQ ID NO:1.

3. The process as claimed in [one of the claims 1 or 2] claim 1, wherein the alkaline phosphatase gene sequence corresponds to SEQ ID NO:5.
4. The process as claimed in [one of the claims 1 to 3] claim 1, wherein methylotrophic yeast cells are used.
5. The process as claimed in [one of the claims 1 to 4] claim 1, wherein *Pichia pastoris* or *Hansenula polymorpha* is used as the yeast strain.
6. The DNA sequence according to SEQ ID NO:5.
7. A transformation vector containing SEQ ID NO:5.
8. The vector as claimed in claim 7, which essentially corresponds to pHAP10-3.
9. A vector [containing] comprising the [entire] expression cassette from pHAP10-3.
10. The vector as claimed in claim 9, which essentially corresponds to pHAP10-3/9K.
11. A host strain transformed with a vector as claimed in claim 9 or 10.
12. A host strain transformed with the vector pHAP10-3/9K and/or a vector as claimed in claims 7 or 8.

13. The host strain as claimed in claim 12, wherein *Pichia pastoris* or *Hansenula polymorpha* is used as the host strain.
14. A *Pichia pastori* X-33 yeast strain transformed with a vector as claimed in claims 8 to 10.
15. A process for producing a eukaryotic highly active alkaline phosphatase, [wherein] comprising the step of expressing the enzyme [is expressed] in a host strain as claimed in one of the claims 11 to 14.

Claims

1. A process for the production of a eukaryotic alkaline phosphatase in yeast cells comprising the steps: a) cloning an alkaline phosphatase gene sequence into different vectors b) transformation of the yeast, c) expression of the alkaline phosphatase and d) purification of the alkaline phosphatase, wherein
 - a first vector is utilized that has a resistance gene for a first selection marker,
 - transformants are used which have integrated the resistance gene and the gene sequence into the genome, said transformants being selected by growth on nutrient medium containing a low concentration of a first selection marker,
 - the gene copy number is increased by multiple transformation in which multiple transformants are selected by growth on nutrient medium at an increased selection pressure,
 - a second vector is used which has a resistance gene for a second selection marker,
 - the gene copy number is increased by multiple transformation with the second vector in which multiple transformants are selected by growth on nutrient medium at an increased selection pressure and
 - those transformants which have integrated several copies of the gene sequence and the selection marker resistance genes in a stable manner are selected for producing the eukaryotic alkaline phosphatase.
2. The process according to claim 1, wherein the gene sequence for the alkaline phosphatase corresponds to SEQ ID NO:1.

3. The process as claimed in claim 1, wherein the alkaline phosphatase gene sequence corresponds to SEQ ID NO:5.
4. The process as claimed in claim 1, wherein methylotrophic yeast cells are used.
5. The process as claimed in claim 1, wherein *Pichia pastoris* or *Hansenula polymorpha* is used as the yeast strain.
6. The DNA sequence according to SEQ ID NO:5.
7. A transformation vector containing SEQ ID NO:5.
8. The vector as claimed in claim 7, which essentially corresponds to pHAP10-3.
9. A vector comprising the expression cassette from pHAP10-3.
10. The vector as claimed in claim 9, which essentially corresponds to pHAP10-3/9K.
11. A host strain transformed with a vector as claimed in claim 9 or 10.
12. A host strain transformed with the vector pHAP10-3/9K and/or a vector as claimed in claims 7 or 8.
13. The host strain as claimed in claim 12, wherein *Pichia pastoris* or *Hansenula polymorpha* is used as the host strain.

14. A *Pichia pastori* X-33 yeast strain transformed with a vector as claimed in claims 8 to 10.
15. A process for producing a eukaryotic highly active alkaline phosphatase, comprising the step of expressing the enzyme in a host strain as claimed in one of the claims 11 to 14.